Inhibition of endogenous APC activity exacerbates thrombo-inflammation in sickle mice at steady state

Kailyn Lowder[1], Nirupama Ramadas PhD[1], Laurent Mosnier PhD[2], Erica Sparkenbaugh PhD[1]

1: Department of Medicine, Blood Research Center, UNC Chapel Hill

2: Department of Molecular Medicine, The Scripps Research Institute

THE UNIVERSITY of NORTH CAROLINA at CHAPEL HILL

INTRODUCTION

- SCD is the most common inherited hemoglobinopathy worldwide
  - Single nucleotide mutation for the β-globin causing sickling of red blood cells (RBCs)
- Key characteristics of SCD:
  - Hemolytic anemia
  - Vascular stasis (1)
  - Hypercoagulability
  - Increased venous thrombosis risk (2)
- Thrombin-dependent PAR1 signaling (R41) activates Gαq and Gα12/13 signaling
  - Promotes endothelial barrier permeability, cytotoxicity, and inflammation (3-4)
- Activated protein C (APC) dependent PAR1 signaling (R46) activates β-arrestin-dependent signaling
  - Anti-inflammatory and cytoprotective (5)
  - Cleaves at a lower affinity than thrombin (6)
  - SCD patients have reduced protein C levels and activity (7)
  - APC has beneficial effects in mouse models of sepsis, stroke, and autoimmune encephalitis (8-10)

AIM

To investigate the role of endogenous APC in SCD.

METHOD

Townes wild type (HbAA) and sickle (HbSS) mice (3-4 months) were treated with control IgG or SPC-54 (10 mg/kg, IP).

- Blood was collected 24 hours after antibody administration
- Total blood cell count and hematologic profile was determined
- ELISAs were used to quantify plasma levels:
  - IL-6, TAT, sP-sel, sVCAM-1
- Lungs and livers were stained:
  1. H&E and scored for congestion and hepatocyte necrosis
  2. For neutrophils and quantified in 10 high power fields
- One-way and Two-way ANOVAs were performed with Tukey's post-hoc test

RESULTS

Parameter	AA/IgG	AA/SPC-54	SS/IgG	SS/SPC-54
RBC (10^6/μL)	9.33 ± 0.19	7.09 ± 0.85*	5.05±0.32****	6.40 ± 0.06*
Hgb (g/dL)	9.56 ± 0.34	7.5 ± 0.93*	6.08 ± 0.41***	8.53 ± 0.24##
Hematocrit (%)	31.8 ± 0.88	24.1 ± 3.02*	22.92 ± 1.69**	31.85 ± 0.72##
Platelets(10^3/μL)	895 ± 23.7	213 ± 16.3****	814 ± 63.7	258 ± 18.0****
WBC (10^3/μL)	8.07 ± 0.74	9.21 ± 0.78	29.63 ± 6.1**	23.21 ± 2.1*
Neut (10^3/μL)	0.98 ± 0.05	5.45 ± 0.76*	6.87 ± 2.19***	12.54±1.11****##
Lymph (10^3/μL)	6.79 ± 0.70	3.49 ± 0.37	22.01 ± 3.9***	10.21 ± 1.19##
Neut:Lymph	0.15 ± 0.14	1.63 ± 0.34***	0.28 ± 0.05	1.29 ± 0.17****##
Mono (10^3/μL)	0.21 ± 0.03	0.24 ± 0.04	0.57 ± 0.12*	0.35 ± 0.07

Table 1. Complete blood counts from HbAA and HbSS mice treated with IgG or SPC-54. *p<0.05, *p<0.01, *p<0.001, *p<0.0001 vs AA/IgG; ##p<0.01 vs SS/IgG. RBC – red blood cell; Hgb – hemoglobin; WBC – total white blood cell count; neut – neutrophil; lymph – lymphocyte; mono - monocyte.

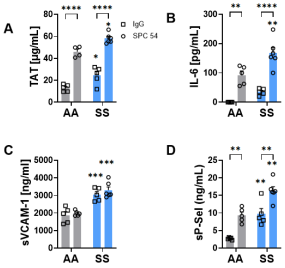

Figure 1. Effect of APC inhibition on markers of thrombin generation, inflammation and endothelial activation in HbAA and HbSS mice. APC inhibition increased TAT (A), IL-6 (B), and sP-sel (D) levels, but did not increase sVCAM1 (C) in both HbSS and HbAA mice. Asterisks above bars indicate difference from AA/IgG. Asterisks above brackets indicate comparisons. *p<0.05, *p<0.01, *p<0.001, *p<0.0001 by Two Way ANOVA and Tukey's Post-hoc test.

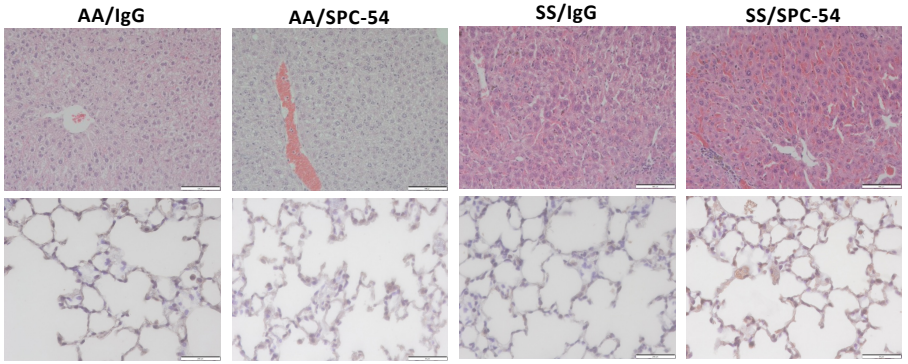

Figure 2: Histological evaluation of liver and lung. Top Row: Representative liver sections stained with H&E and assessed for vascular congestion and necrosis. Bottom Row: Representative lung sections stained for neutrophils (PMNs) (400X magnification).

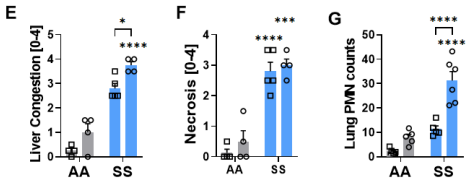

Figure 3. Effect of APC inhibition on organ pathology. APC inhibition exacerbated the liver congestion (A) but not hepatocyte necrosis (B). APC inhibition increased the number of PMNs the lungs of HbSS mice (C). Asterisks above bars represent comparison to AA/IgG, whereas asterisks above brackets indicate comparison. *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001 by Two Way ANOVA and Tukey's Post Hoc Test.

CONCLUSIONS

We found that APC inhibition with SPC-54 increased TAT, IL-6, and soluble p-sel in HbAA mice, and further exacerbated the already elevated levels of these markers in HbSS mice.

In HbAA mice, APC inhibition RBC and platelet counts in HbAA mice compared to IgG-treated controls, but modestly increased circulating RBC parameters in HbSS mice. APC inhibition also significantly increased circulating neutrophil and neutrophil:lymphocyte ratios in both HbAA and HbSS mice, indicating that APC inhibition enhances acute inflammatory responses.

Histologic evaluation of the livers of SPC-54-treated HbSS mice revealed enhanced vascular and sinusoidal congestion that contained both RBCs but also inflammatory cells, but APC inhibition did not increase hepatic necrosis.

This suggests that endogenous APC plays an important role in mitigating thrombo-inflammation in SCD.

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CONTACT INFORMATION

Kailyn Lowder
Blood Research Center, Division of Hematology
116 Manning Dr, Chapel Hill, NC 27599
University of North Carolina Chapel Hill
Email: klowd@email.unc.edu